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THE EFFECT OF DONOR AGE ON ENGINEERED TENDON TISSUE DERIVED FROM TENOCYTES AND MESENCHYMAL STEM CELLS

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Purpose: To compare age-related changes in the proteome of tendon tissue-engineered constructs (TEC) derived from mesenchymal stem cells (MSCs) and tenocytes.
Methods: Liquid chromatography tandem mass spectrometry was used to assess the whole proteome of TEC derived from young (n = 7) and old (n = 6) equine tenocytes or human MSCs (equal sample sizes, n = 4). Label-free (LF) quantification was undertaken to identify proteins differentially expressed (DE) between the age groups. Structural, functional and protein network analysis of the data was performed using Ingenuity Pathway Analysis (IPA), Matrisome DB and the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) for tenocyte and MSC TEC separately. Neopeptide identification was performed to determine extracellular matrix (ECM) fragmentation patterns. Immunohistochemistry and biochemical analysis were also undertaken.
Results: 205 proteins were considered DE in MSC and 41 in tenocyte-derived TEC. LF quantification demonstrated a higher number of ECM protein, nuclear proteins, and a lower number of cytoplasmatic and plasma membrane proteins in the tenocyte TEC in comparison to MSC TEC. For both cell types ageing was associated with increased abundance of cytoskeletal proteins, including actin-binding molecule calponin 1, considered to be responsible for cell mechanoresponsiveness. Calponin 1 was among the most abundant DE protein determined by proteomic analysis and was confirmed using immunohistochemistry in old MSC and tenocyte TEC. Increased number of neopeptides for collagenous protein was identified in old TEC derived from both types of cells, suggesting enhanced collagen turnover in ECM. Gene ontology terms determined for proteins DE in old MSC TEC were related to increased cell antioxidant response (FDR < 0.001) , whilst in tenocyte TEC to accumulation of collagen breakdown products (FDR < 0.001). Increased total collagen content in old TEC was confirmed using a hydroxyproline assay. Transforming growth factor beta-1 was proposed as potential upstream regulator of age-related changes in ECM composition of tenocyte and MSC-derived TEC.
Conclusions: Ageing affects synthetic activity of cells used for generation of tendon TEC, which is reflected by alterations in their protein content. Proteomic profile of ageing in tendon TECs differs between MSC and tenocyte-derived constructs. Age-related changes in TEC protein composition may affect their functionality and should be taken into consideration in applying autologous cell therapies in elderly patients.

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TARGETING CARTILAGE AGING AS OSTEOARTHRITIS THERAPEUTICS BY DRUG REPURPOSING

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Purpose: Effective treatments for Osteoarthritis (OA) are not available. In aging-related diseases, including OA, failure of cellular homeostasis mechanisms, such as autophagy can cause extracellular matrix destruction and cell death. Chondrocytes are essential for the maintenance of cartilage integrity. With aging, chondrocyte function is diminished, contributing to a cellular senescence phenotype often observed in OA chondrocytes. In addition, a defect in autophagy is observed in both aging and cartilage degeneration. *The objective of this study was to identify anti-senescence and pro-autophagy molecules by a cell-based high-throughput screening (HTS) in human chondrocytes.*
Methods: To induce cellular senescence or reduced autophagy, immortalized human chondrocytes (TC28a2) were seeded (2500 cells/well) in 384 well plates, and treated with IL-6 (20 ng/ml) for 72 or 18 hours, respectively. Then, chondrocytes were incubated with Prestwick Chemical Library (1120 approved drugs with chemical and pharmacological diversity, as well as bioavailability and safety in humans) at 10 μM for 72 hours. To identify anti-senescence hits, nuclei was stained with Hoechst 33342 (2,5μg/ml), while β-galactosidase subcellular structures was stained by using Imagene Green C12FDG substrate (30 μM). To evaluate autophagic flux, a reporter cell line was generated by retrovirus transfection of pBABE-mCherry-EGFP-LC3 plasmid in TC28a2 chondrocytes. Plates were imaged by using Operetta® High Content Screening (HCS) system in non-confocal mode using the 20x WD objective. For each well, 4 fields and 4 planes of bright field, Hoechst and fluorescein channels were obtained. Relative intensity of C12FDG in cytoplasm and number of autophagosomes/autolysosomes per area of cytoplasm were determined to quantitate β-galactosidase activity and autophagy flux respectively. Compound validation was performed in TC28a2 chondrocytes and in primary human chondrocytes by evaluating cell senescence, autophagy pathway and cell death by apoptosis.
Results: A primary screening was performed to identify anti-senescence compounds by measurement of senescence-associated β-galactosidase activity. 308 compounds with anti-senescence effects were identified. The anti-senescence hits were analyzed by monitorizing autophagic flux. 42 compounds with both anti-senescence and pro-autophagy effects were selected. Then, one compound was selected for further validation. The compound reduced chondrocyte senescence, increased autophagy ($p < 0.0001$) and protected against cell death by apoptosis in human chondrocytes ($p < 0.05$) in response to IL-6. Interestingly, this protective effect was partially mediated by mTOR inhibition, a proposed mechanism to prevent cartilage aging.
Conclusions: Our screening methodology provides a unique opportunity to repurpose drugs and mechanisms to prevent cartilage aging. Autophagy activation and protection against senescence in chondrocytes may provide benefits for delaying cartilage degeneration.

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STRAIN-DEPENDENT DIFFERENCES IN AGE-ASSOCIATED OSTEOARTHRITIS PATHOGENESIS

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Purpose: Osteoarthritis (OA) is a progressive disabling disorder affecting the entire joint. In order to elucidate molecular mechanisms and assess interventions, many laboratories use animal models to recapitulate disease progression. The majority of rodent studies of OA employ surgical models to induce a post-traumatic disease phenotype. However, post-traumatic OA only affects a subset of patients, and these results may not be generalized to other types of OA. Similarly, many groups use C57/B6 mice for transgenic studies, and CD1 mice for wild-type in-vivo or ex-vivo studies; yet the spontaneous progression of OA in CD1 mice has not been evaluated. Our first aim was to compare aging models of wild type CD1 and C57/B6 mice to examine whether there are strain-specific differences that could affect the progression of OA. In addition, we previously showed that cartilage-specific inactivation of the nuclear receptor PPARdelta results in significantly decreased severity of OA in a model of post-traumatic OA in mice. Our second objective was to assess whether cartilage-specific knockout of PPARdelta is protective in an aging model of OA (in a C57/B6 background).
Methods: CD1, C57/B6, and cartilage-specific PPARdelta knockout mice were aged to 12 months, 20 months, or 24 months, and then subjected to gait analysis. End point blood glucose and liver weight were measured. Knees, elbows, and ankles were harvested for histology. Mice were compared through classical measures of OA progression including Toluidine-blue staining with OARSI scoring, immunohistochemistry for cartilage matrix breakdown products, picrosirius red staining for collagen structure and organization, as well as quantitative measures of cartilage and synovial thickness. Synovium was scored based on degree of hyperplasia and infiltration of inflammatory cells, and bone changes were assessed through H&E and TRAP staining for osteoclasts.
Results: CD1 mice at 1 year of age present with early OA-like changes to the knee joint synovium and subchondral bone, including thickening of both tissues, but otherwise healthy joints. At 20 months of age CD1 mice have severe OA in the medial compartment of the knee affecting all joint tissues, including cartilage degeneration, marrow changes and subchondral bone thickening, and synovial hyperplasia. This is further exacerbated at 2 years of age, and is more severe in male mice than female mice at all time points. Conversely, C57/B6 mice present with only minor changes in articular cartilage at 2 years,